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Structural analysis of adeno-associated virus transduction circular intermediates.

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Recombinant adeno-associated virus (rAAV) has recently been demonstrated to form circular intermediates following transduction in muscle tissue and cell lines. Although restriction enzyme and Southern blot analysis has revealed a consistent monomer and multimer head-to-tail conformation, detailed structural sequence analysis has been lacking due to the high secondary structure of the ITR arrays. To gain further insight into potential mechanisms by which AAV circular genomes are formed from linear single-stranded viral DNA, we have performed chemical sequencing of ITR arrays within seven circular intermediates independently isolated from primary fibroblasts and Hela cells. Results from these studies demonstrated several types of circular intermediates with mosaic ITR elements flanked by two D sequences. The most predominant form consisted of a structure similar to that of previously generated AAV double-D plasmids, with one complete ITR flanked by two D-region elements. However, intermediately deleted ITR arrays with more than one complete ITR were also seen. Based on this structural information, we have proposed a model for formation of AAV circular intermediates by recombination/ligation between ITR ends of panhandle single-stranded AAV genomes.

PMID: 10484751 [PubMed - indexed for MEDLINE]

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